BRAF Mutations: The Discovery of Allele- and Lineage-Specific Differences

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Cancer treatment is increasingly guided by molecular analyses designed to identify clinically actionable genomic alterations in individual patients. The discovery of *BRAF* mutations in human cancer, and the subsequent development and FDA authorization of selective BRAF inhibitors highlight the potential clinical impact and current limitations of precision oncology paradigms. In 2002, Brose and colleagues reported that the distribution of BRAF mutations differed in melanoma and lung cancer and that not all BRAF mutations were functionally equivalent. Here, we discuss this landmark paper, which foreshadowed subsequent research elucidating how biochemical differences among mutant alleles within the same gene and lineage-specific differences among cancer types impact drug sensitivity. Such translational studies provided a road map for the development of novel RAF inhibitors and rational combination strategies that promise greater clinical activity and/or more favorable toxicity profiles.

See related article by Brose and colleagues, Cancer Res 2002;62:6997–7000

The RAS–RAF–MEK–ERK (MAPK) pathway is a key mediator of cell growth and survival in normal and cancer cells. A causative link between dysregulated MAPK signaling and cancer dates to the 1960s with the identification of the RAS proto-oncogene as the sarcoma-inducing factor in several oncogenic murine retroviruses. Although early attempts to directly inhibit mutant RAS were unsuccessful, a series of publications in 2002 re-centered the narrative of targeting dysregulated MAPK signaling on inhibition of the BRAF serine/threonine protein kinase. In the first paper, Davies and colleagues used automated capillary sequencing technology (high throughput Sanger sequencing) to analyze the coding sequence and intron–exon junctions of *BRAF* in DNA derived from 923 human cancer cell lines and tumors (1). They identified BRAF mutations in approximately 8% of samples including in 66% of melanomas. *BRAF* mutations were concentrated in the kinase domain, with 80% a single valine to glutamic acid hotspot mutation at codon 600 within the activation segment (BRAF V600E, initially mischaracterized as V599E). Rescreening of a large subset of the cohort revealed four tumors with coincident RAS and BRAF mutations, all of which were non-V600 mutations. In an elegant experiment, the authors microinjected a RAS-neutralizing mAb into BRAF mutant and wild-type cancer cell lines to inhibit RAS signaling, finding that proliferation was undisturbed only in the nine cell lines harboring a BRAF V600E/D mutation. This study foreshadowed the creation of large-scale consortia, such as the Tumor Cancer Genome Atlas (TCGA), to molecularly profile large cohorts of patient tumors to identify additional oncogenic mutations that could serve as drug targets.

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As all four BRAF mutations identified by Davies and colleagues in lung cancers were non-V600E, many of the same investigators subsequently published in *Cancer Research* a genomic analysis of an expanded cohort of lung cancers and melanomas to better define the prevalence of BRAF mutations and the distribution of BRAF mutant alleles in these two common cancer types (2). They observed a significant difference in both the frequency of BRAF mutations between cancer types (3% of lung cancers vs. 63% of melanomas) and the distribution of BRAF mutant alleles (one V600E and four non-V600 mutations in lung cancer vs. 21 V600 and two non-V600 mutations in melanoma). The authors speculated that the BRAF mutants found in melanoma and lung cancer were qualitatively different, with potential implications for the future development of BRAF selective inhibitors.

Differences in BRAF mutation frequency and allele distribution among cancer subtypes have since been validated by the TCGA and prospective large-scale clinical sequencing initiatives designed to accelerate enrollment into genotype-directed clinical trials of novel cancer drugs (3). As shown in **Fig. 1A**, the frequency of BRAF alterations varies widely among cancer types, with the highest prevalence of oncogenic and likely oncogenic BRAF mutations in hairy cell leukemia (nearly 100%), papillary thyroid cancer (70%), cutaneous and unknown primary melanomas (42%), and Langerhans cell histiocytosis (39%). BRAF mutations are less common, but of clinical significance, in colon cancer (10%) and non–small cell lung cancer (4%). BRAF V600E is by far the RAF mutation most often detected in human cancers, with alternate codon 600 substitutions (V600K/R/D/L) infrequently observed (**Fig. 1B**). Additional oncogenic non-V600E BRAF mutations localize to the activation segment (near V600) and the glycine-rich phosphate-binding loop (residues 464–469) of the kinase domain. Activating in-frame deletions (e.g., L485-P490del and exon 2–10 RAS binding domain deletions) and BRAF fusions are also observed in a minority of cancers. As initially observed by Brose and colleagues, the distribution of BRAF mutant alleles varies widely among cancer types, with codon 600 mutations representing over 70% of BRAF mutations in melanoma, but only 30% of BRAF mutations in non–small cell lung cancer and 1% of BRAF mutations in prostate cancer (**Fig. 1B**). Mutations and gene fusions involving ARAF and CRAF are observed at much lower frequencies in human cancer, likely due to biological differences among the three RAF isoforms. Most

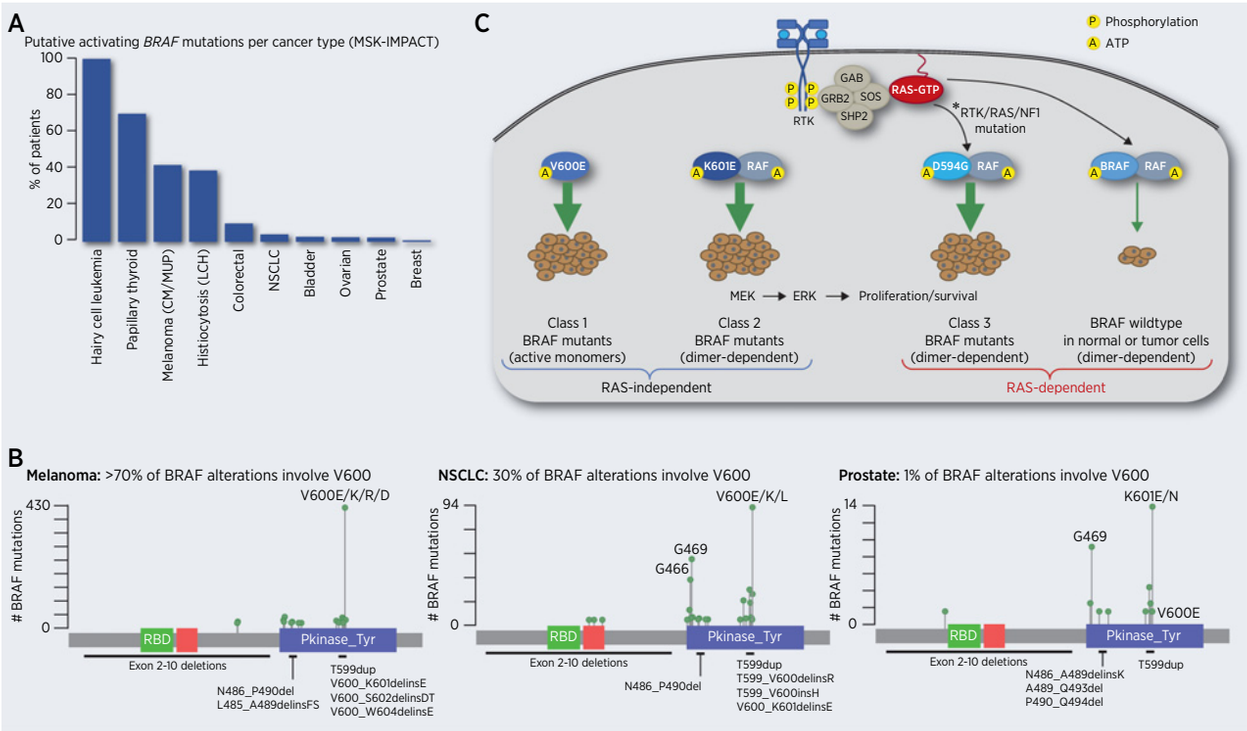

Figure 1.

Landscape of *BRAF* mutations in human cancer and mechanisms of mutant BRAF activation. **A,** Frequency of *BRAF* mutations in ten cancer subtypes curated from the prospective MSK-IMPACT genomic sequencing initiative (>75,000 samples). The prevalence of BRAF mutations varies across cancer types. CM, cutaneous melanoma; LCH, Langerhans cell histiocytosis; MUP, melanoma of unknown primary. **B,** Distribution and frequency of putative oncogenic BRAF mutations and in-frame insertion/deletions in melanoma, non–small cell lung cancer (NSCLC), and prostate cancer in the MSK-IMPACT cohort. Oncogenic BRAF fusions (not depicted) are also found in a minority of tumors. **C,** Simplified model of MAPK pathway activation by wild-type BRAF and class 1, 2, and 3 BRAF mutants. Wild-type BRAF and class 3 mutants, but not class 1 or class 2 mutants, rely on upstream RAS input for activity. Only class 1 BRAF mutants can signal as active monomers.

notably, BRAF, in contrast to ARAF and CRAF, has a phosphomimetic residue (D448) and a constitutively phosphorylated residue (S445) in its negatively charged N-region near the C-terminus, which allows a single kinase domain mutation to render the protein constitutively active.

Detailed mechanistic studies over the past two decades have led to the classification of BRAF mutants into one of three classes based on the mechanisms by which they activate ERK signaling (4, 5). Class 1 and class 2 BRAF mutants function independently of RAS but with therapeutically relevant differences: class 1 mutants can signal as active monomers (V600 mutants), whereas class 2 mutants signal as active dimers (e.g., K601E, BRAF fusions/splice variants, among others). Class 3 mutants, which typically have impaired kinase activation or are kinase dead, promote MAPK activation cooperatively with RAS and thus are often co-occurrent with RAS mutations, NF1 mutation/deletion, or mutations/amplification of upstream receptor tyrosine kinases (**Fig. 1C**; ref. 6).

The translational importance of these biochemical distinctions lies in the differential sensitivity of the BRAF mutant classes to FDA-approved and investigational RAF inhibitors. In the mid-2000s, our group showed that BRAF V600E mutant cell lines and xenografts are exquisitely sensitive to MEK inhibition (7). MEK inhibitors are now FDA-approved for the treatment of BRAF V600E melanomas, where they are often used in combination with RAF inhibitors. However, MEK inhibitors potently inhibit MAP kinases signaling in both tumor and normal tissues, leading to sometimes intolerable, on-target toxicities such as skin rash. In 2011, vemurafenib, a selective RAF inhibitor, was FDA-approved for the treatment of patients with late-stage melanoma harboring the BRAF V600E mutation. Although vemurafenib binds to all three RAF isoforms and both wild-type and mutant BRAF, it only inhibits MAPK signaling in tumors with codon 600 BRAF mutations. Conversely, in normal cells and in many tumors with non-V600 BRAF mutants or wild-type BRAF including those with RAS mutations, vemurafenib increases MAPK signaling, which can result in toxicity and accelerated tumor progression (8). The basis for the BRAF V600 mutant selectivity of vemurafenib and its paradoxical activation of MAPK signaling in normal cells are mechanistically linked. Biochemical studies revealed that the BRAF V600E mutant can signal as a monomer that can be inhibited by vemurafenib. In contrast, vemurafenib binding to one RAF protomer within RAF dimers induces negative allostery, which sterically reduces the affinity of vemurafenib for the second RAF protomer (9). This leaves the dimer partner transactivated and primed to activate MEK. As a result, vemurafenib is ineffective at inhibiting MAPK signaling in cancer cells, in which the MAPK pathway is activated by class 2 and 3 BRAF mutants or upstream alterations such as RAS mutations, NF1 loss, or receptor tyrosine kinase activation.

In addition, in contrast to the nearly universal sensitivity of patients with BCR-ABL–driven chronic myelogenous leukemia to ABL kinase inhibitors, a substantial fraction of BRAF V600E tumors are

intrinsically resistant to vemurafenib, with the likelihood of response varying widely as a function of tumor lineage (response rates of >80% in BRAF V600E histiocytosis, 50% to 60% in BRAF V600E melanoma, and less than 5% in BRAF V600E colorectal cancer). Acquired drug resistance to RAF inhibitors is also the rule rather than the exception, with only a minority of patients having durable responses lasting greater than one year. Consistent with its mechanisms of mutant selectivity, laboratory and clinical studies have now shown that drug resistance to vemurafenib is often the result of alterations that induce RAF dimer formation including RAS mutations, NF1 loss, and the expression of BRAF splice variants that lack the RAS binding domain (10). Nonmutationally mediated adaptive resistance can also result from relief of ERK-induced negative feedback inhibition of upstream receptor tyrosine kinases, such as EGFR in patients with colorectal cancer.

To address the intrinsic resistance of BRAF non-V600 mutant tumors to vemurafenib and to overcome intrinsic and acquired drug resistance in BRAF V600E mutant tumors, ongoing drug development efforts are being directed towards the identification of drugs that inhibit RAF dimers or do not induce MAPK signaling in normal cells. Promising agents include RAF inhibitors that are equipotent against mutant RAF monomers and dimers (e.g., BGB659) and "RAF dimer breakers," which are designed to inhibit BRAF V600 monomers and disrupt dimeric BRAF mutants but spare the RAF dimers that mediate MAPK activation in normal cells (e.g., PLX8394; ref. 11). Another strategy to overcome RAF inhibitor resistance is to employ novel combinations that more potently inhibit MAPK signaling (vertical pathway cotargeting) or inhibit co-occurent mutations and alternate pathways that reduce BRAF dependence (oncogenic bypass). An example of the former is cotreatment with an inhibitor of SHP2, a nonreceptor protein tyrosine phosphatase scaffold for SOS1, which could attenuate feedback reactivation of RAS and reduce the formation of RAF dimers. Finally, combinations of RAF inhibitors with immunotherapies such as vemurafenib, cobimetinib, and atezolizumab (anti-PD-L1 antibody) have recently demonstrated longer progression-free survival than targeted therapy alone (12). Together with the development of novel molecular profiling approaches that can identify epigenetic and other non-DNA–based mechanisms of treatment resistance, there is optimism that there will soon be more effective and less toxic treatments available for a larger proportion of patients with BRAF mutant tumors.

Authors' Disclosures

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